

RESEARCH ARTICLE

A dynamical model for characterising seasonality effects on eelgrass plastochron intervals

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Abstract

The plastochron interval is widely used to calculate age and rates of productivity in many plants, including seagrasses. However, plant responses to changing environmental conditions, including seasonal patterns, can introduce substantial errors in methods for calculating age and rates of growth. We propose a generalised method for characterising seasonal variability in eelgrass plastochron values based on a model that consists of a linear combination of a trend, a seasonality component and a stochastic noise component. The model was validated using data obtained biweekly during 1998–2003 in a *Zostera marina* meadow in a coastal lagoon in northwestern Baja California. Plastochron intervals exhibited marked interannual and seasonal variability as well as in the timing of plastochron interval (PI) minima and maxima. Correlation analyses indicated that sea surface temperature is a fundamental forcing factor for the plastochron interval, whose local variability is influenced by the onset of ‘El Niño’ and ‘La Niña’ events. The proposed model provided reliable interpretations that elicited the existence of seasonal processes which are usually masked by multimodal changes in the plastochron interval. Using successive averages of seasonal PI to describe annual cycles resulted in reliable leaf-growth assessments as well as in better determinations of shoot age than those calculated using a single annual mean.

Introduction

Askenasy (1880) defined a plastochron as the time interval elapsed between the formation of two successive internodes in *Nitella flexilis*. He suggested that the term might be used for all plants with a similar nodal organisation. Indeed, the concept has been used extensively, particularly in shoot-apex studies of higher plants in which leaves appear periodically. This period of initiation has been termed a plastochron or plastochron interval (Esau, 1953; Lamoreaux *et al.*, 1978). Erickson & Michelini (1957) noted that when successive plastochrons are equal in duration, the elapsed time may be made to serve as a unit of a developmental scale. This unit was termed a plastochron index (Lamoreaux *et al.*, 1978). The notion of a plastochron interval was first related to studies of seagrasses by Patriquin (1973), who presented

a formula for the estimation of the time interval between the establishment of two successive leaves in *Thalassia testudinum*. The output values were used to determine plant age as well as for the calculation of rates of growth and production of below-ground parts. Determinations of plastochron interval for *Zostera marina* were initially reported by Sand-Jensen (1975) and formally defined by Jacobs (1979) as the number of marked shoots multiplied by the observation period in days divided by the number of new leaves produced (Eqn 1). Values of the PI calculated with this formula were used to obtain production rates of shoots, leaves, roots and rhizomes.

The ascendancy of the PI as a valuable tool in seagrass research has been confirmed by further applications. For instance, in demography, mean annual PI has been considered as a unit in a chronological age scale and has been used to determine age of shoots (Jacobs, 1979;

Brouns, 1985; Duarte & Sand-Jensen, 1990a,b; Duarte, 1991; Gallegos *et al.*, 1992; Kraemer & Alberte, 1993; Duarte *et al.*, 1994; Gallegos *et al.*, 1994; Marbá *et al.*, 1994; Van Tussenbroek, 2002). The suitability of the plastochron interval (as well as other structural and shoot-specific characteristics) as an ecological indicator was evaluated by Durako (1995). In addition, in metabolic and physiological measurements, samples have been standardised in terms of the PI to reduce within-treatment variation (Dennison, 1980). Kraemer & Alberte (1993) used the PI to correlate the age of *Z. marina* tissue with total below-ground and above-ground biomass as well as with indices of metabolic capacity such as respiration, soluble carbohydrate content and glutamine synthetase activity. Gacia *et al.* (2003) showed that mineral load in seagrass leaves increases with age and used the PI to determine this variable. Moreover, generalised plastochron-related methods have also been used for measuring growth in many species based on the time interval between the production of new plant parts and the size of a mature leaf (Short & Duarte, 2001).

For seagrass species, the average annual rate of leaf formation has been widely considered a reliable proxy for PI values. Annual rates of leaf formation are usually expressed on a per day basis and are calculated using annual averages of leaf formation divided by 365 days. This amounts to assuming that leaf formation occurs evenly throughout the year. However, it has been shown that changes in structural characteristics of seagrasses, including mean annual PI, reflect responses to environmental conditions (Durako, 1995; Solana-Arellano *et al.*, 2009). Indeed, leaf production in seagrasses shows seasonal patterns even in tropical environments (Brouns, 1985; Van Tussenbroek, 1994, 1995; Gaeckle & Short, 2002), and the coupling of seasonal processes to PI dynamics has led to methodologies that use seasonal signals imprinted in the sizes of seagrass modules to determine PI values (e.g. Duarte & Sand-Jensen, 1990b; Pergent & Pergent-Martini, 1990; Gallegos *et al.*, 1992). The effects of plastochron interval variability in related estimation methods have been stressed by several authors. For example, Marbá *et al.* (1996) showed that the use of dating techniques to resolve shorter-than-annual time scales involves greater error because the PI in temperate seagrasses varies seasonally. Kaldy *et al.* (1999) showed that the reliability of plastochron methods for estimating short shoot age in *T. testudinum* was limited by substantial interannual, seasonal and site-specific variability. Moreover, Gaeckle & Short (2002) stated that because the plastochron interval varies seasonally, in order to use the plastochron method to estimate leaf growth, the PI must be measured for each growth determination. Variability in PI values might thus introduce

substantial errors in methods for estimating age and growth and production in seagrasses.

Sand-Jensen (1975) and Jacobs (1979) stated that under normal conditions, eelgrass plastochron intervals are shorter during summer-autumn periods (July through December) and longer in the winter-spring season (January through June). To distinguish between seasonal and non-seasonal processes, Duarte (1999) fitted a second-order polynomial. Significant fits were considered to indicate seasonality, whereas lack of fit suggested non-seasonal growth patterns. In this study, however, fitting a second-order polynomial to observed PI values resulted in a poor correspondence between predicted and observed values for different annual cycles, and the complete time series of observed PI values displayed a complex multimodal pattern. Nevertheless, in our view, these facts would not necessarily imply that seasonal processes should be disregarded. Rather, we suggest that their elucidation might be resolved on the basis of an appropriate formalisation of a suitable paradigm for the dynamics of eelgrass growth.

A conceptual framework for eelgrass growth must consider the widespread belief that seasonal variations in light and temperature fundamentally regulate productivity in temperate seagrasses. This has been sustained by the results of correlation analyses reported in several studies (e.g. Sand-Jensen, 1975; Jacobs *et al.*, 1982; Phillips *et al.*, 1983; Wium-Andersen & Borum, 1984; Evans *et al.*, 1986; Kentula & McIntire, 1986; Orth & Moore, 1986; Olesen & Sand-Jensen, 1994; Lee *et al.*, 2004). Furthermore, any attempt to characterise the effects of seasonality must also take into account other results which demonstrate that seagrasses growing in similar latitudes can display a wide range of seasonal responses (e.g. Kaddy & Patriquin, 1978; Alcoverro *et al.*, 1995; Marbá *et al.*, 1996). In addition to seasonal influences, factors such as local variation in environmental variables can also significantly affect growth in these species (Dennison & Alberte, 1986; Evans *et al.*, 1986; Pirc, 1986; Alcoverro *et al.*, 1995; Masini *et al.*, 1995; Marbá *et al.*, 1996; Cebrián *et al.*, 1997). In particular, local environmental influences on eelgrass growth include anoxia, current speeds and wave exposure, desiccation, epiphyte and grazer load, grazing by water fowl, nutrient limitations and toxicity, and sediment grain size (Tennant, 2006 and references therein). In addition, reactions to large-scale influences might be modulated by species-specific responses to environmental forcing. These species-specific effects may result from different physiological responses to light and temperature conditions (Evans *et al.*, 1986) as well as from other endogenous factors, such as the ability to allocate resources to production or the differential ability of the species to store and use resources (Ashmun *et al.*, 1982;

Pitelka & Ashmun, 1985; Marbá *et al.*, 1996). As an example of eelgrass species-specific responses, we may refer to the ability of this plant to modify its growth form in response to environmental conditions. Populations experiencing low frequencies of disturbance predominantly allocated biomass to increasing shoot size and maintaining stable densities of shoots (Olesen & Sand-Jensen, 1994). Such species-specific effects could partially uncouple plant growth from environmental conditions (Dennison & Alberte, 1986; Evans *et al.*, 1986; Pirc, 1986; Williams, 1988; Masini *et al.*, 1995; Marbá *et al.*, 1996; Lee *et al.*, 2003). However, seasonal responses of seagrasses may, nevertheless, be set by endogenous rhythms that could render seasonal dynamics relatively independent of interannual environmental variability (Ott, 1979; Pergent, 1990; Kaldy *et al.*, 1999). In this contribution, we propose a generalised method for the characterisation of changes in eelgrass PI values that take into account the confluence of dynamical influences mentioned above. Its formal foundation is a model comprising a trend component, a seasonal component and a stochastic noise component. Our results show that the proposed device provides a reliable analytical tool that elicits the existence of seasonal processes which are usually masked by multimodal changes in the plastochron interval.

Materials and methods

Study site

Data used for this study were collected in a *Z. marina* meadow at Punta Banda estuary, a shallow coastal lagoon located near Ensenada, Baja California, Mexico (31°43–46'N and 116°37–40'W). The climate is characterised by warm, dry summers and cool, moist winters. Evaporation normally exceeds precipitation, but extreme winter storms can drive freshwater input to exceed evaporation (Acosta-Ruiz & Alvarez-Borrego, 1974; Celis-Cecena & Alvarez-Borrego, 1975). This makes the estuary a permanent hypersaline environment where tides control water renewal in a fundamental way. Depth decreases from the mouth [12.5 m mean low water (MLW)] to the head (1 m MLW) of the estuary, whereas water temperature and salinity increase. The residence time of water decreases from the mouth towards the head. Currents are tidally driven and generally $<0.15 \text{ ms}^{-1}$ (Pritchard *et al.*, 1978). Water transparency decreases consistently towards the head (Millan-Núñez, 1981). Relatively cold and nutrient-rich waters that upwell in the adjacent California Current region are carried to the area contiguous to the mouth of the estuary, and tidal currents transport these waters to the interior. Remineralisation of nutrients in sediments, and turbulence induced by tidal

currents and winds, increase nutrient concentrations in surface waters (Millan-Núñez, 1981).

Data processing

We sampled the site biweekly from April 1998 through September 2003. At every sampling, approximately 40 shoots were marked (Kentula & McIntire, 1986), and the previously marked shoots were removed. The shoots collected were placed in a portable cooler and transported to the laboratory for processing. We determined both the total numbers of leaves present and the number of new leaves among them. Plastochron interval values $PI(t)$ associated with a marking date t were calculated using the formula:

$$PI(t) = \frac{R_S(t) \Delta t}{NL_S(t)}, \quad (1)$$

where $R_S(t)$ is the number of recovered shoots, Δt is the number of days between marking and recovery of shoots and $NL_S(t)$ denotes the number of new leaves produced during the interval (Jacobs, 1979). For each one of the collected shoots, following the approach described in Jacobs (1979) and addressed by Gaeckle & Short (2002), we identified a representative fully formed leaf. This is generally the third leaf counting from the youngest to the oldest in the bundle of leaves in a shoot. The biomass of these third leaves was then used to obtain assessments of leaf-growth rates based on PI; these are denoted here by means of the symbol $Lgs_3(t)$ and calculated according the formula,

$$Lgs_3(t) = \frac{ws_3(t)}{PI(t)}, \quad (2)$$

where $ws_3(t)$ stands for the weight of the third leaf in the considered shoot.

Data were analysed using the STATISTICA (2007) program. For 1999 to 2001, data were collected on complete rhizomes and used to estimate shoot age according to the reconstruction method of Duarte *et al.* (1994). Shoot age is calculated by adding the number of leaf scars (or internodes) plus the number of standing leaves produced. The resulting age estimations are expressed in plastochron intervals. The number of plastochrons for each shoot was then translated to days using average PI values obtained by leaf-marking procedures.

Sea surface temperature (SST) and photosynthetically active radiation (PAR) were measured at the site using a Quantum Radiometer–Photometer (Li-Cor, Inc., Lincoln, NE, USA) with an integration time of 1000 s. Concentrations of nutrients [phosphates (P) and nitrates (N)] were measured in samples taken biweekly from the water column, but only from November 1999 through

June 2001. Nutrient concentration was determined by flow injection analysis at the UC Santa Barbara Marine Science Institute. We also performed a correlation analysis among variables including the El Niño Southern Oscillation (ENSO) multivariate index (MEI) and the oceanic El Niño index (ONI). The MEI attempts to monitor ENSO events on the basis of six main variables observed over the tropical Pacific: sea-level pressure, zonal and meridional components of the surface wind, SST, surface air temperature and total cloudiness fraction of the sky. Negative values of the MEI represent the cold ENSO phase (La Niña), whereas positive MEI values represent the warm ENSO phase (El Niño), <http://www.esrl.noaa.gov/psd/people/klaus.wolter/MEI/>. Correspondingly, the ONI is defined as the 3-month running average of SST anomalies in the El Niño 3.4 region (5°N–5°S, 120–170°W). An El Niño occurs when ONI is at least 0.5°C for at least five consecutive trimesters and conversely, La Niña conditions occur when ONI reaches –0.5°C or less for five consecutive seasons.

Modelling tools

One might expect that the confluence of factors that determine seagrass growth would result in such an intricate diversity of influences as to make the selection of a functional model a daunting task. Experience gained in other fields, however, confirms that one can rely on an empirical approach, and we focus on the views of Marbá *et al.* (1996), who proposed a paradigm that views the seasonality of growth in seagrasses as primarily determined by two components: an extrinsic component dependent on seasonal forcing by light and temperature and an endogenous (species-specific) component. This endogenous component likely involves a differential capacity of the species to regulate its own internal resource economy, which may buffer, or amplify, the external seasonal forcing. Similarly, we assert that the dominance of the endogenous component can render seasonal dynamics relatively independent of environmental variability. In *Z. marina*, this endogenous component of variability is likely determined by a gamut of processes which cannot be clearly distinguished by analysing month-to-month growth parameters (Palacios & Zimmerman, 2007). It is considered that such cyclical variables can only be unambiguously identified in a time series that is at least three times longer than the period expected (Duarte *et al.*, 1994). Characterising the subtleties in PI dynamics by formal methods would thus require a data set collected over a suitably long observational period (Kaldy *et al.*, 1999; Estes & Peterson, 2000). In addition to the data set requirements, we would also need a model that includes dynamical components

in accordance with the aforementioned paradigm for seagrass growth. We therefore must consider an empirical dynamical model that includes three fundamental components. First would be an explicit representation of seasonality. Second, one must necessarily take into account unpredictable effects that might influence the strength of the seasonal component. These could be because of variability associated with large-scale seasonal forcing as well as the effects of different endogenous responses. A convenient way to represent such effects could be by adding a stochastic term to the seasonal component. Finally, large-scale influences associated with systematic effects such as global climate change could also induce trends in growth. We may thus consider that the dynamics of seagrass growth can be formally expressed as a linear combination of a trend, a seasonality component and a stochastic noise component.

The proposed approach for modelling seagrass growth can be adapted to eelgrass PI dynamics in a straightforward way. We can refer to Marbá *et al.* (1996), who also presented an outstanding account of the responses of seagrass PI dynamics to environmental variability. They asserted that seagrass response to climate change may be constrained by the rate of addition of modules and that the dynamics of PI values will limit the capacity of plants to respond to environmental variability. Furthermore, the clonal growth of eelgrass (McMillan, 1982; Reusch *et al.*, 1999) makes module growth closely related to PI dynamics (Patriquin, 1973; Brouns, 1985; Gaeckle & Short, 2002). In fact, expanding modules are formed by rhizome segments with bundles of attached leaves and associated roots. Because every leaf produced corresponds to the production of a rhizome node, it is reasonable to assume that eelgrass growth and leaf formation are equivalent processes (Duarte *et al.*, 1994). Moreover, it can be asserted that the extent to which clonal patterns of eelgrass growth are altered in response to environmental disturbances will determine both the magnitude and the number of deviations from a normal pattern of PI variation. Hence, we may suppose that eelgrass PI variability can also be expressed in terms of the dynamical components named above.

To provide an explicit representation for the proposed model, we introduce a notational and methodological device aimed at describing different expressions of seasonality in observed PI(*t*) values. Towards that aim, we partition the complete yearly cycle into *m* disjoint and equally sized periods, each of π months. These periods will be called seasons and each one of these partitions will be called a π -seasonal partition for short. For consistency, we must have the relationship

$$m\pi = 12. \quad (3)$$

Then, with each π -seasonal partition, we associate a model $PI_{\pi}(t)$ of the form:

$$PI_{\pi}(t) = T_{\pi}(t) + S_{\pi}(t) + e_{\pi t}, \quad (4)$$

where $T_{\pi}(t)$ stands for a trend term, $S_{\pi}(t)$ represents what we call a π -seasonality component and $e_{\pi t}$ is a random variation remainder or error.

We assume that, in general, $T_{\pi}(t)$ can be represented through the n th degree polynomial,

$$T_{\pi}(t) = \sum_{k=1}^n a_{\pi k} t^k, \quad (5)$$

where, for each fixed value π and for $0 \leq k \leq n$, the $a_{\pi k}$ entries are constants. Meanwhile, the π -seasonality component will be assumed to have the form,

$$S_{\pi}(t) = \sum_{k=1}^m c_{\pi k} Q_{\pi k}(t), \quad (6)$$

where for fixed π and $1 \leq k \leq m$, the $c_{\pi k}$ entries are constants and $Q_{\pi k}(t)$ are indicator variables associated with the seasons determined by the π partition. Moreover, the $Q_{\pi k}(t)$ variables are codified by means of 1 or 0 values. A value of 1 pertains to a given time t in a particular season, otherwise a 0 value is attained. Finally, the errors are assumed to be serially correlated; that is, $e_{\pi t}$ satisfies

$$e_{\pi t} = \rho_{\pi} e_{\pi t-1} + v_{\pi t}, \quad (7)$$

where ρ_{π} stands for a constant called the strength of the serial correlation and $v_{\pi t}$ is the stochastic noise.

Results

The dynamics of PAR and SST are shown in Fig. 1a and the concentrations of N and P in Fig. 1b. Similarly, the dynamics of PI values measured are shown in Fig. 2. All of these plots display marked interannual variability. Considerable variability also occurred in the timing of PI minima and maxima (Table 1), with maxima unexpectedly occurring during the cold seasons and minima during the warm seasons of 1998, 1999 and 2002. These results occurred in a manner opposite to results previously reported by Sand-Jensen (1975) and Jacobs (1979). Correlation analysis showed that *in situ* SST anomalies, the MEI and the ONI values all had significant correlations with the observed PI values [(-0.57, $P < 0.02$), (-0.42, $P < 0.0010$) and (-0.45, $P < 0.001$), respectively]. All these variables were also found to be highly correlated with each other, with correlations ranging from 0.65 to 0.84 ($P < 0.05$ for all) (Solana-Arellano *et al.*, 2009). The correlation coefficient between plastochron interval and luminosity was only of

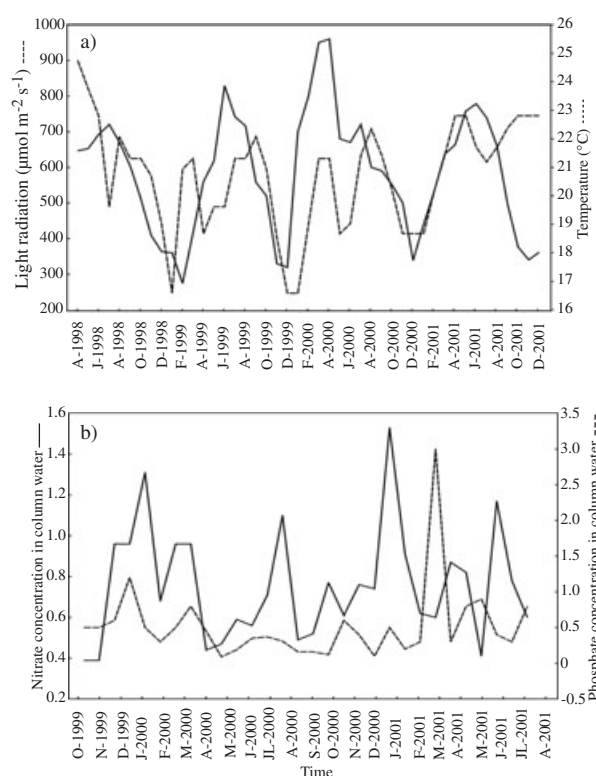


Figure 1 (a) Changes in light radiation (continuous line) and sea surface temperature at the study site (dashed line). (b) Nutrient concentrations in the water column: nitrate (continuous line) and phosphate (dashed line). Only dates for which measurements of these variables are available are shown.

$r = -0.10$. Taken together, these results suggest that SST can be considered a fundamental environmental forcing factor for the plastochron interval.

The distribution through time of the observed PI values was roughly that of an upward-facing quadratic curve, and so we assumed that the trend term $T_{\pi}(t)$ could be represented in the form

$$T_{\pi}(t) = a_{\pi 0} + a_{\pi 1}t + a_{\pi 2}t^2. \quad (8)$$

Meanwhile, to characterise Eqn (6), we produced different π -seasonal partitions of a whole-year cycle. A first partition ($\pi = 2, m = 6$) corresponds to 1/6-year periods, with the first one being January and February, with indicator variable $Q_{21}(t)$, and the last one November and December, with indicator variable $Q_{26}(t)$. We called these periods first to sixth 2-month seasons, respectively. Similarly, the partition ($\pi = 3, m = 4$), ($\pi = 4, m = 3$) and ($\pi = 6, m = 2$) with indicator variables $Q_{31}(t)$ through $Q_{34}(t)$, $Q_{41}(t)$ through $Q_{43}(t)$ and $Q_{61}(t)$ through $Q_{62}(t)$, respectively, divided the year into 1/4, 1/3 and 1/2-year seasonal periods, respectively; the first one from January to March, with indicator variable $Q_{61}(t)$, and the last one

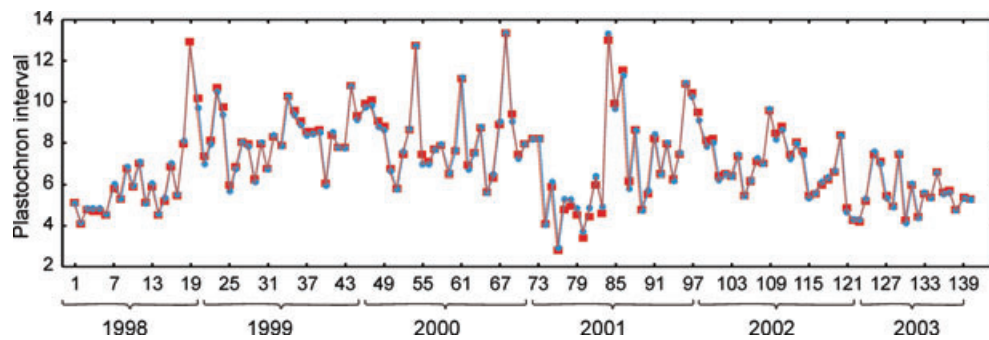


Figure 2 Comparison of observed and predicted plastochron index values. Continuous blue lines correspond to observed values, and dashed red lines show values predicted by the proposed model (cf. Eqns 3–8).

Table 1 Statistics for plastochron intervals for the years sampled and months when minima and maxima were attained

Year	Mean PI (days)	Minimum PI (days)	Maximum PI (days)	SD
1998	6.0	4.1 (May)	12.8 (December)	1.9
1999	8.1	5.6 (March)	10.8 (December)	1.4
2000	8.3	5.6 (October)	13.4 (August)	1.9
2001	6.8	2.9 (March)	13.3 (June)	2.6
2002	7.2	5.3 (September)	10.2 (January)	1.37
2003	5.5	4.1 (May)	7.5 (March)	1.05

PI, plastochron index.

from October to December, with indicator variable $Q_{61}(t)$. We called these periods first to fourth seasons of each year.

Once the $Q_{\pi k}(t)$ were codified, for each value of π , we considered that the associated model $PI_{\pi}(t)$ were fitted with the trend $T_{\pi}(t)$ term given by Eqn (8) and the form of Eqn (6) determined by the choosing of π . The resulting representation of Eqn (4) was fitted to observed $PI(t)$ data using non-linear least-square estimation. The model of Eqn (7) was then fitted to the resulting $e_{\pi t}$ values in order to corroborate serial correlation in the residuals (Wonnacott & Wonnacott, 1984). Among the π -seasonal partitions, the case $\pi = 6$ produced the most consistent fit (Table 2). In all these fittings, the associated $v_{\pi t}$ were found to be normally distributed with a mean of 0 and constant variance. A stepwise test for each of the $PI_{\pi}(t)$ models showed that the observed PI values are mainly described by a dominant half-year seasonal component. Fig. 2 shows the progression of observed and predicted values through time. In order to provide an additional model selection criterion, we performed for each π -seasonal partition a two-way analysis of variance for the PI variable seeking differences among years and periods. We used a factorial design of order two and a type III (orthogonal) analysis of variance model. Table 3 shows

Table 2 Fitted parameters for Eqns (6) and (8) for all π -partitions^a

π	R^2	ρ_{π}	Durbin-Watson d -Value	Tendency	Seasonality
2	0.84	0.66	1.1	$a_{\pi 1} = 0.23$ ($p < 0.005$) $a_{\pi 2} = -0.0015$ ($p \leq 0.005$)	$c_{21} = 2.09$ ($p = 0.44$) $c_{22} = 0.15$ ($p = 0.81$) $c_{23} = 0.01$ ($p = 0.98$) $c_{24} = 1.4$ ($p = 0.05$) $c_{25} = -0.52$ ($p = 1$) $c_{26} = -0.52$ ($p = 1$)
3	0.91	0.26 ($p < 0.005$)	2.05	$a_{\pi 1} = 0.09$ ($p \leq 0.005$) $a_{\pi 2} = -0.0007$ ($p = 0.30$)	$c_{31} = 4.5$ ($p < 0.005$) $c_{32} = 4.8$ ($p < 0.005$) $c_{33} = 5.5$ ($p < 0.005$) $c_{34} = 5.3$ ($p < 0.005$)
4	0.94	0.42 ($p < 0.005$)	1.2	$a_{\pi 1} = 0.07$ ($p < 0.005$) $a_{\pi 2} = -0.0005$ ($p < 0.005$)	$c_{41} = 5.6$ ($p < 0.005$) $c_{42} = 5.9$ ($p < 0.005$) $c_{43} = 6.0$ ($p < 0.005$)
6	0.97	0.40 ($p < 0.005$)	1.17	$a_{\pi 1} = 0.07$ ($p < 0.005$) $a_{\pi 2} = -0.0005$ ($p < 0.005$)	$c_{61} = 5.5$ ($p < 0.005$) $c_{62} = 6.2$ ($p < 0.005$)

^aThe d -value gives the autocorrelation of residuals. If $d < 2$, there is evidence of positive serial correlation. Conversely, if $d > 2$, then successive error terms are negatively correlated. No autocorrelation is indicated if $d = 2$.

the significant differences among years and semesters found in the analysis, thus corroborating a half-year seasonality.

For the plastochron method (Jacobs 1979; Gaeckle and Short, 2002) for the estimation of leaf growth based on PI, we found that 6-month seasonal plastochron estimations produced projections of leaf-growth rates that were similar to those calculated using monthly and annual plastochron averages. The time series of produced mean shoot ages expressed in plastochrons is shown in Fig. 3. In order to assess the effects of plastochron variability, we produced shoot-age estimations in days.

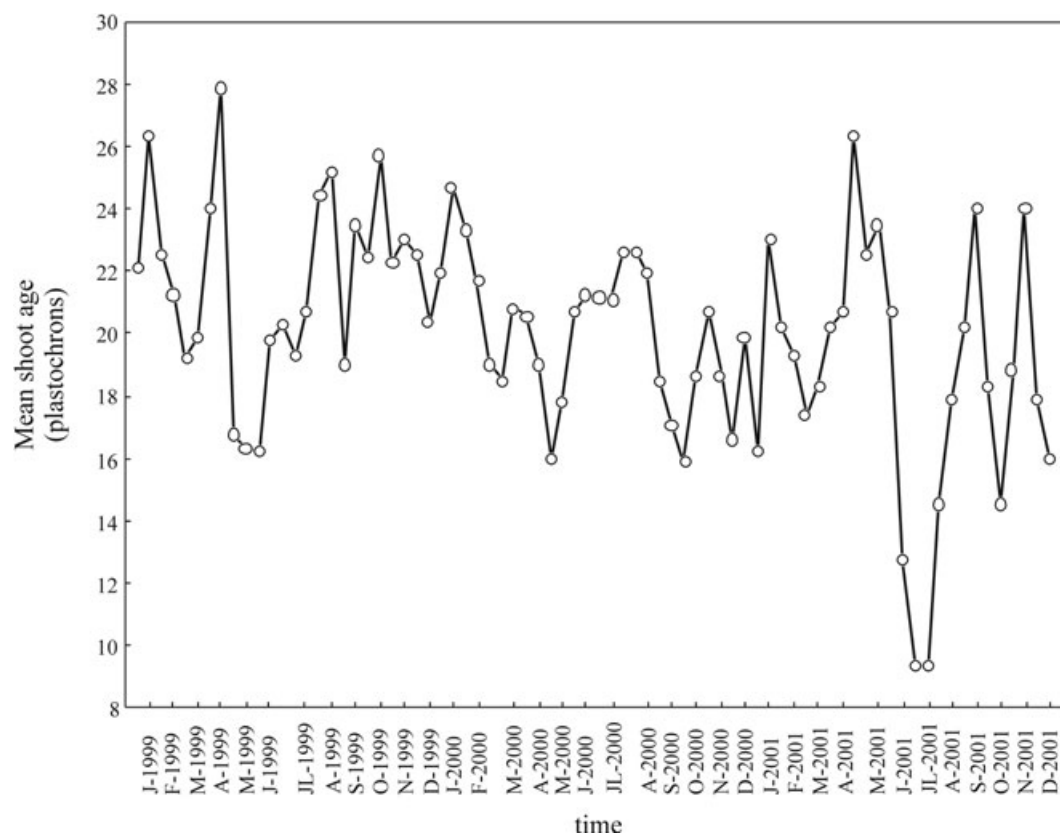
Table 3 Results from the two-way analysis of variance for each period^a

Effect	Mean Square	Degrees of Freedom	F	p-Value
Year	20.4	5	5.1	<0.05
$\pi = 2$	9	5	1.8	0.20
Interaction	4.1	22	1.6	0.07
Residual	2.6	107		
Year	24.4	5	4.9	<0.05
$\pi = 3$	9.3	3	1.9	0.18
Interaction	4.8	14	1.64	0.08
Residual	2.9	117		
Year	26.5	5	8.3	<0.05
$\pi = 4$	0.5	2	0.17	0.84
Interaction	3.7	8	1.2	0.31
Residual	3.1	124		
Year	84.4	5	10.1	<0.05
$\pi = 6$	65.7	1	6.5	<0.05
Interaction	8.4	5	3.0	<0.05
Residual	2.8	128		

^aMean squares and *F* values are rounded off to decimals. The table shows significant differences for the plastochron index variable for $\pi = 6$.

These were based on average PI values taken over biweekly, monthly, half-year seasonal and annual periods which we called reference, monthly, half-year seasonal

and annual PI estimations, respectively. An inspection of the estimated ages shows that for small shoots (ages from 0 to 100 days), the observed monthly and half-year seasonal plastochron interval overestimates reference ages by an average of 29.5% and 30.2%, respectively, whereas mean annual PI overestimated reference age assessments by about 40%. For medium shoots (aged from 100 to 150 days), reference values were overestimated by about 7% and 8% compared with monthly and half-year seasonal PI averages and by about 13% compared with annual PI averages. Large shoots (aged above 150 days) were underestimated 7%, 9% and 12% using monthly, half-year seasonal and annual PI averages. Using seasonal PI averages always produced estimated shoot ages that were closer to the reference ones than those obtained using annual PI averages. Moreover, a mean-root-square deviation value between reference and half-year seasonal estimates was smaller than either monthly or annual assessments. Thus, using successive averages of half-year seasonal plastochron interval to describe a whole-year cycle resulted in better shoot-age determinations than those calculated from a single annual mean.

**Figure 3** Pictorial representation of estimated ages (in plastochrons) using the Duarte *et al.* (1994) reconstructive method.

Discussion

ENSO as disturbance factor

In this oceanographic region, adjacent to the northwestern Baja California peninsula, environmental influences on eelgrass growth are significantly controlled by climatic and oceanographic features characteristic of the California Current system. Disturbances are mainly associated with warm 'El Niño' events and resulting changes in salinity, modifications in upwelling processes and anomalously high sea-level values (Durazo & Baumgartner, 2002). We can assume that the availability of dissolved nutrients at our study site was largely influenced by upwelling, as was the case in a nearby coastal lagoon studied by Álvarez-Borrego (2004), so the onset of an ENSO event would also be expected to significantly reduce nutrient availability at the Punta Banda estuary. It is well known that changes in sea level can influence the availability of light in the water column for seagrass growth (Short & Neckles, 1999). Hence, besides irradiance, colinearity effects suggest that SST can be considered to be a factor that integrates the fundamental environmental forcing for the measured PI values (Solana-Arellano *et al.*, 2009).

The hypothesis that variation in PI values is linked primarily to changes in SSTs is supported by the results of Reusch *et al.* (2005), who found that during periods of high water temperatures (20–25°C), *Z. marina* ceases growth and reduces shoot density up to 50%. A similar conclusion was obtained by Lee *et al.* (2006), who reported that SST was fundamental in the determination of eelgrass growth. Their results might explain the occurrence of anomalous timing of maxima and minima in our PI data and also suggest that the observed multimodal pattern could have resulted from species-specific responses to changes in the normal range of SST induced by 'El Niño' and 'La Niña' events.

Sea surface temperature forcing and anoxia

Hartman & Brown (1976) demonstrated that the oxygen produced by photosynthesis is stored and recycled in the internal lacunal system of macrophytes. Radial diffusion plays an important role in transporting oxygen stored in the lacunal system to the intercalary meristem (Sand-Jensen *et al.*, 2005). Moreover, O₂ concentration in the meristem is closely coupled to O₂ concentrations in the overlying water column (Greve *et al.*, 2003; Pedersen *et al.*, 2004). Thus, temperature-driven anoxia can be considered as an important effect that might result in significant forcing on local environmental variability. Low oxygen concentrations, due to elevated temperatures in the water column, are not unusual in natural seagrass beds and are often accompanied by severe mortality

(Frederiksen & Glud, 2006). Dissolved oxygen levels vary seasonally in estuaries, with the lowest levels occurring in late summer when temperatures are highest (Odum & Odum, 1959; Yin *et al.*, 2004). Maintenance of oxic conditions in meristematic and below-ground tissues of seagrasses is an important support for rapid growth, nutrient uptake by roots and translocation of nutrients and carbohydrates between roots and leaves (Smith *et al.*, 1988; Zimmerman & Alberte, 1996). The availability of dissolved oxygen is also an important factor controlling the production of new leaves (Greve *et al.*, 2003) because of the high oxygen demand in the meristem to support cell division and growth (Brix & Sorrell, 1969). Hence, diminished oxygen availability can be expected to play an important role in controlling PI dynamics. This linkage between the availability of dissolved oxygen and the production of new leaves in eelgrass likely provides an explanation for the SST forcing that we found.

Irradiance

The SST was found to be dominant over irradiance in explaining the PI dynamics, which deserves further elaboration. It is widely believed that light radiation is the most important factor that determines seagrass seasonality (e.g. Backman & Barilotti, 1976; Zimmerman *et al.*, 1987; Olesen & Sand-Jensen, 1993; Duarte, 1995; De Casabianca *et al.*, 1997; Moore *et al.*, 1997; Havens *et al.*, 2001; Nelson & Lee, 2001; Watanabe *et al.*, 2005). Nevertheless, Olsen & Sand-Jensen (1994) reported that a severe reduction in eelgrass leaf biomass prior to the onset of growth enhanced light availability and the survival of new shoots, thereby contributing to the recovery of the populations following major disturbances. This species-specific response implies that longer PI values could be expected during low irradiance conditions. During our study period, nearby dredging only altered irradiance during a few short and sporadic episodes. This seems to indicate that low PI values in our data could have been caused by factors other than low irradiance. A similar conclusion was reached by Burkholder *et al.* (1992) for an eelgrass population in Chesapeake Bay. Moreover, shallow distribution of plants could have facilitated direct exposure to such an extent that even during low irradiance episodes, light was not a limiting factor for growth. This could explain why light radiation was not found to be a dominant factor for PI variation in the studied population.

Species-specific responses

The length of the PI in seagrasses is considered to be a strong species-specific feature (Duarte, 1991). For eelgrass, in particular, such responses to

environmental influences manifest primarily through phenotypic plasticity (McMillan & Phillips, 1979; Backman, 1991; Rhode & Duffy, 2004), and thus the eelgrass PI can be included in the set of responses to stressful and changing environments (e.g. Marbá *et al.*, 1996; Guidetti, 2000; Hemminga & Duarte, 2000; Brun *et al.*, 2006; Billingham *et al.*, 2007). A typical PI value for eelgrass is 13.1 days (Duarte, 1991), so in comparison with species with a longer interval, eelgrass would be expected to display a fast response to seasonal fluctuations (Marbá *et al.*, 1996). Hence, the large number of turning points in the multimodal pattern observed suggests that the variability in PI likely resulted from a combination of both large-scale environmental influences, including disturbances, and phenotypic plasticity responses linked to an endogenous component of variability. In spite of these marked fluctuations, however, a persistent dynamic prevailed which indicates the dominance of the seasonal component. Thus, the observed multimodal PI dynamics likely corresponds to a prevailing seasonal growth pattern that was masked by deviations modulated by species-specific responses to disturbances. This effect could have been driven by an endogenous component that rendered the manifestation of seasonality relatively independent of environmental variability (Ott, 1979).

Significance of plastochron interval seasonality in estimations of leaf growth and shoot age

For leaf-growth determinations obtained by means of the plastochron method, we found that half-year seasonal plastochron estimations produced projections of leaf-growth rates that were similar to those calculated using monthly and annual plastochron averages. Gaeckle & Short (2002) stated that to use the plastochron methods, the PI must be measured for each growth determination. However, our results show that PI values determined over half-year seasonal periods can produce equivalent eelgrass growth estimations. Thus, a prior determination of the PI seasonal variation mode can simplify data-gathering procedures. This adds to the benefits of an elucidation of a masked seasonality in a PI variation pattern. Moreover, the usefulness of the seasonality component was confirmed by focusing on average shoot-age and leaf-growth estimations. Annual PI averages are commonly used to scale age in days from plastochrons. The consideration of half-year seasonal PI averages always produced estimated shoot ages that were closer to the reference (biweekly) ones than those obtained using annual PI averages.

Our results confirm that the determination of eelgrass PI seasonality provides new insights into estimations of eelgrass leaf growth and shoot age. Nevertheless,

this determination of seasonality cannot be achieved by a direct interpretation of variability. The identification of the model defined by Eqn (4) provided a reliable interpretative tool. Moreover, the dummy-variables method defined by Eqn (6) unravelled the underlying seasonality processes in a consistent way; that is, the assumption of serial correlation of errors provided a convenient description of unpredictable changes in dynamics. This demonstrates that the combination of an informative observational scale and a proper formalisation of a suitable paradigm for growth can elicit the existence of seasonal processes that are masked in multimodal PI dynamics.

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